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RESEARCH ARTICLE

# Assessment of bioaerosol contamination (bacteria and fungi) in the largest urban wastewater treatment plant in the Middle East

**Sadegh Niazi[1,2] · Mohammad Sadegh Hassanvand[1,2] · Amir Hossein Mahvi[1,3] · Ramin Nabizadeh[1,2] · Mahmood Alimohammadi[2] · Samira Nabavi[2] · Sasan Faridi[2] · Asghar Dehghani[4] · Mohammad Hoseini[2] · Mohammad Moradi-Joo[4] · Adel Mokamel[2] · Homa Kashani[5] · Navid Yarali[5] · Masud Yunesian[1,2]**

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**Abstract** Bioaerosol concentration was measured in wastewater treatment units in south of Tehran, the largest wastewater treatment plant in the Middle East. Active sampling was carried out around four operational units and a point as background. The results showed that the aeration tank with an average of 1016 CFU/m$^3$ in winter and 1973 CFU/m$^3$ in summer had the greatest effect on emission of bacterial bioaerosols. In addition, primary treatment had the highest impact on fungal emission. Among the bacteria, *Micrococcus* spp. showed the widest emission in the winter, and *Bacillus* spp. was dominant in summer. Furthermore, fungi such as *Penicillium* spp. and *Cladosporium* spp. were the dominant types in the seasons. Overall, significant relationship was observed between meteorological parameters and the concentration of bacterial and fungal aerosols.

**Keywords** Air contamination · Bioaerosols · Wastewater treatment plant · Tehran

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Sadegh Niazi and Mohammad Sadegh Hassanvand are coequal first author.

✉ Masud Yunesian
niazsadegh@gmail.com

1 Center for Air Pollution Research (CAPR), Institute for Environmental Research (IER), Tehran University of Medical Sciences, Tehran, Iran

2 Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

3 Center for Solid Waste Research (CSWR), Institute for Environmental Research (IER), Tehran University of Medical Sciences, Tehran, Iran

4 Cancer Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

5 Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

## Introduction

Wastewater treatment plants (WWTP) could affect the environmental health in many different ways. These effects depend on the size of wastewater treatment plant, technology, and treatment methods (Sánchez-Monedero et al. 2008). Wastewater contains many pathogens such as viruses, bacteria, fungi, protozoa, and helminthes which originate from human activities in household, commercial, and other institutions. These microorganisms can easily become airborne during the process of treatment at WWTP (Carducci et al. 2000). Bioaerosol is one of the most important contaminants in WWTP, which may include various types of microorganisms. Bioaerosols are generated at different stages of wastewater treatment process, particularly in process that containing moving mechanisms and performed aeration of wastewater (Pascual et al. 2003). Bioaerosol particles (bacteria and fungi) exist naturally in the air or derived from living organisms. Fungal spores and bacteria typically have diameters between 1–30 μm and 0.25–8 μm, respectively (Bredholt et al. 2008). However, atmospheric air due to lack of nutrients does not provide favorable condition for growth of microorganisms, but they could be present in aerosol form in the air (Kruczalak and Olanczuk-Neyman 2004). Survival of airborne microorganisms in aerosols depend on several environmental factors such as radiation ultraviolet, temperature,

humidity and pressure, the type of microorganisms, and some pollutant availability in the atmosphere (Cox and Wathes 2010). The potential hazard caused by bioaerosols is related to pathogenicity of specific microorganisms, environmental conditions, exposure pathway, and immunologic response of the host (Burkowska et al. 2012). In WWTP, the highest emission of bioaerosols occurs in pretreatment and primary clarifiers units and those containing moving mechanical equipment for wastewater aeration (Pascual et al. 2003). It has been found that most of the bacteria-carrying particles in the air of a WWTP had an aerodynamic diameter below 4.7 μm; hence, they can easily enter the lung and cause infections in immunocompromised individuals and allergic reaction (Recer et al. 2001). Endotoxins produced by gram-negative bacteria are of concern which may lead to airways and intestinal inflammation, diarrhea, nose irritation, fever, and fatigue in sewage treatment plant workers (Oppliger et al. 2005; Thorn et al. 2002). Particular form of a disease, known as *wastewater workers syndrome*, has been found among wastewater treatment workers (Basart et al. 2012). Because of scarce information in employee's hazards exposure in this WWTP, this study was conducted aiming to (1) determine the fungal and bacterial concentration, (2) identify the fungi and bacteria genera, and (3) investigate the effects of seasons and different parameters on the bioaerosol amounts in South Tehran Wastewater Treatment Plant.

## Material and methods

### Sampling sites

The study was performed in Tehran wastewater treatment plant which is located in the southeast of Tehran, the capital of Iran. The main characteristics and design parameters of the WWTP are described in Table 1. Sampling stations were selected according to the location of units, wind direction, and the proximity of workers. The sampling points are described in Table 2.

### Sampling methods

Sampling was carried out in both warm and cold seasons during December 2012 to August 2013. According to EPA sampling guideline, sampling was done in every 6 days; in the warm and cool season, 24 samples were collected from each station and a total of 240 samples were obtained for bacteria and fungi (Hoseini et al. 2012). Air sampling was performed for 2 min using QuickTake 30 sample pump equipped with the Bio Stage single-stage cascade impactor (SKC, USA). The pump was set at flow rate of 28.3 L/min, and the height of sampling was 1.5 m (respiratory height) (Mentese et al. 2012). The flow rate of pump was calibrated by a manometer. After sterilization with alcohol (70 %), the culture medium was placed inside Biostage (Abdel Hameed et al. 2009). Also, meteorological conditions including atmospheric temperature, humidity, wind direction, and pressure were measured at each sampling location (Table 3).

Tryptic soy agar (TSA) was used as the transfer culture medium to examine airborne bacteria. In addition, Saboraud dextrose agar (SDA) was applied to transfer media for airborne fungi (Lee and Jo 2006; Wang et al. 2010). In order to prevent fungal growth, the cyclohexamide antibiotic was added to bacterial culture media when the temperature had fallen to approximately 47 °C after autoclaving; also, chloramphenicol antibiotic was used to suppress any bacterial growth in fungal culture medium (Korzeniewska 2011a, b; Kim et al. 2009). The agar medium was prepared in laboratory and transferred under sterile condition to WWTP. After sample collecting, culture media were placed in zip kips and transferred to the laboratory in cool box (Abdel Hameed et al. 2009).

**Table 1** Main characteristics and design parameter of the wastewater treatment plant

| | |
|---|---|
| Number of inhabitant served | 2100000 |
| Number of site workers | 250 |
| Design flow ($m^3$/day) | 449280 |
| Maximum flow ($m^3$/h) | 28080 |
| Aeration system | Air diffusion by fine bubble diffusers |

### Identification of bacterial and fungal bioaerosols

To identify bacterial spices, the plates were placed in an incubator at temperature of 35°±0.5 °C for 24–48 h (Scaltriti et al. 2007). Then, colonies on each plate were counted and reported as colony-forming unit (CFU/$m^3$), while the bacterial genera were identified according to Bergey's manual and biochemical tests (Kim et al. 2007, 2009; Faridi et al. 2014). The fungal media were transferred to the laboratory, and airborne fungi were cultured for 3–7 days at room temperature (20–25 °C). The simple method of slide culturing was established to identify the fungal species by performing some levels of microscopic study by using optical microscopes (Karra and Katsivela 2007; Faridi, et al., 2014 ).

### Data analysis

Experimental data were analyzed by SPSS software version 20. One sample Kolmogorov-Smirnov test was performed to determine the normality of the data. Then, Pearson correlation coefficient and regression model was used to find the correlation between bacteria, fungal detected, and meteorological conditions.

**Table 2** Description of locations and sampling points

| location/sampling point | Description |
|---|---|
| A | Adjacent the aeration tank and secondary sedimentation units, 15 m below the aeration unit |
| B | Near the tricking filter, at a 15-m distance the tricking filter |
| C | Near the sludge storage tank and sludge dewatering unit, at a 15-m distance the dewatering unit |
| D | Adjacent the screening, grit chamber, and primary sedimentation unit, at a 15-m distance primary sedimentation unit |
| Background | Outside WWTP, upstream wind direction |

## Result and discussion

### Bacterial concentration

Figure 1 presents the concentrations of airborne bacteria in the five sampling stations. As depicted in Fig. 1, the average of bacteria produced in location A (adjacent the aeration tank) was higher than other locations. The mean concentrations of detected bacteria in the warm and cold seasons were 1973 and 1016 CFU/m$^3$, respectively. The results have shown that the major factors in emission of bioaerosols in the WWTP were: (a) turbulence and tremor in wastewater, (b) wind speed and direction and wind effect level, and (c) rainfall (Michałkiewicz et al. 2011). In contrast to mechanical aeration, the diffusion aeration system undergoes less turbulence. However, in the aeration unit of this WWTP because of high wind effect and secondary settling along the unit, the emission of bacterial and fungal aerosols seems to be in high level. In addition, in location D (Adjacent the screening, grit chamber and primary sedimentation unit), the rate of emission was to be high. In other words, the rates of bacteria in this location were found to be 1882 CFU/m$^3$in the summer and 904 in the winter. This condition appears to have been a consequence of using sludge collectors, high level of wind effect, and presence of grit chambers and screening. In locations B (near the tricking filter) and C (near the sludge storage tank and sludge dewatering unit), the emission rate was low due to less turbulence and low wind effect. A few studies showed that mechanical mixing of the wastewater aiming to aerations leads to increased bioaerosols in the air (Brandi et al. 2000; Breza-Boruta and Paluszak 2007). Once the resulted particles fall down again, they are broken into even smaller particles with a diameter of 50–100 μm as the hit surface of wastewater, causing secondary pollution. These particles are rapidly evaporated in the air and their aerodynamic diameter become smaller in such a way that they cannot settle but remain in suspension (Filipkowska et al. 2000). In similar studies reported by Michałkiewicz et al. 2011, Wlazło et al. 2001, Oppliger et al. 2005, and Fernando and Fedorak 2005, likewise in the present study, aeration tanks were found to be the major point in emitting bioaerosols. In the contrary, Karra recognized aeration-based gritting chamber as the main source of bioaerosol emission (Karra and Katsivela 2007; Heinonen-Tanski et al. 2009; Laitinen et al. 1994) reported the maximum degree of pollution was caused by pretreatment and gritting chamber.

### Concentration of airborne fungi in different sampling locations

Figure 2 shows the rate of fungal aerosol emission based on CFU/m$^3$ around the four sampling units and the background. As illustrated in Fig. 2, location D contain the highest rate of emission in the winter and in the summer with values of 781 CFU/m$^3$ and 1063 CFU/m$^3$, respectively, followed by location A with an emission average of 705 in the winter and 944 CFU/m$^3$ in the summer. Also, the minimum rate of fungal emission was seen in location C. In a similar study, the grit chamber was found to be the most important cause of fungal emission (Kim, Kim et al. 2009). Furthermore, Pascual found that pretreatment was the most effective point in fungal emission (Pascual et al. 2003).

### Airborne bacterial identification

The factors affecting amounts and types of air microbial condition are various and dependent to organic/mineral materials, particulates, temperature, geographical location, moisture, rainfall, and others (Oppliger et al. 2005) The active growth of bacteria is sensitive to dryness and destroyed easily. This sensitivity is even more in gram-negative bacteria comparing gram-positive bacteria. In other words, gram-positive bacteria and fungal spores are more tolerant to dryness, consequently can stay more in the air (Fang et al. 2005). In this study, most of the detected bacteria were gram-positive bacteria.

**Table 3** Mean of meteorological conditions

| Season | Temperature (°C) | Wind speed (km/h) | Pressure (hPa) | Humidity (%) | UV index |
|---|---|---|---|---|---|
| Winter | 7.16 | 10.8 | 903.3 | 50.4 | 4 |
| Summer | 30 | 11.1 | 898.3 | 29.4 | 9.5 |

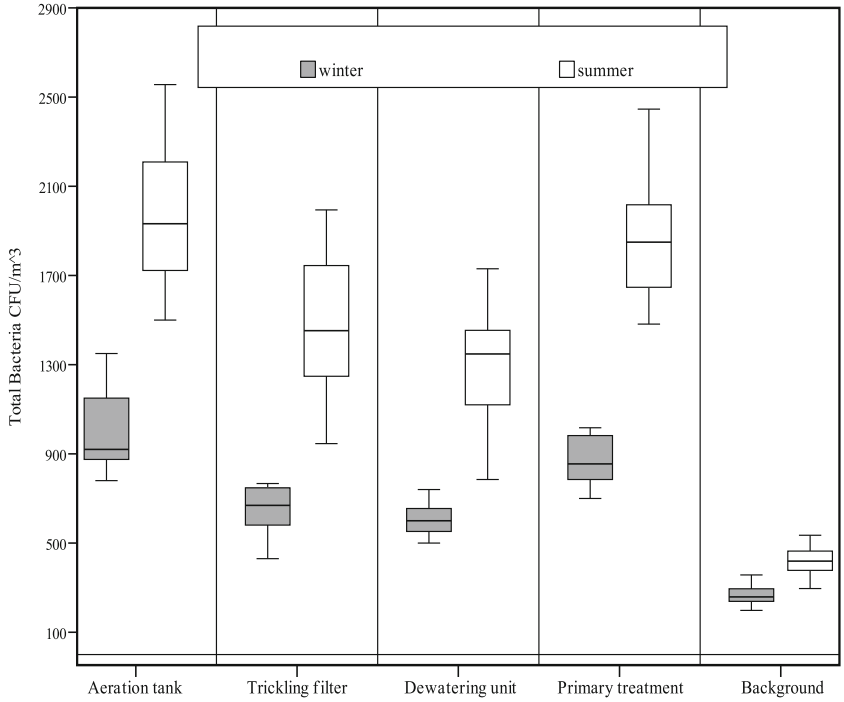


**Fig. 1** Concentration of detected bacteria in sampling point in two seasons (winter and summer) as CFU/m$^3$ ($N$=120)

Numerous studies showed that the concentration of bioaerosols in WWTPs depends on the sampling location (Breza-Boruta and Paluszak 2007), type of microorganism, type of wastewater, aeration method, climatic conditions, wastewater treatment equipment, sunlight, wind speed, and relative humidity (Karra and Katsivela 2007; Michałkiewicz et al. 2011). These bioaerosols survive even after traveling thousands of miles and can cause infection (Papke and Ward 2004). Table 4 shows the concentration of bacteria species that were found in the five locations in two different seasons. In winter, the highest rate of emission was *Micrococcus* spp., whereas in summer, *Staphylococcus* spp. showed the highest rate. Emission of *Bacillus* spp. was relatively constant over the two seasons. Table 4 illustrates the frequency of types and species of the bacteria observed in the study. The distribution of bacteria in the two seasons was approximately steady in all of the sampling locations except in some cases that types and species had more distribution. According to Table 4, in

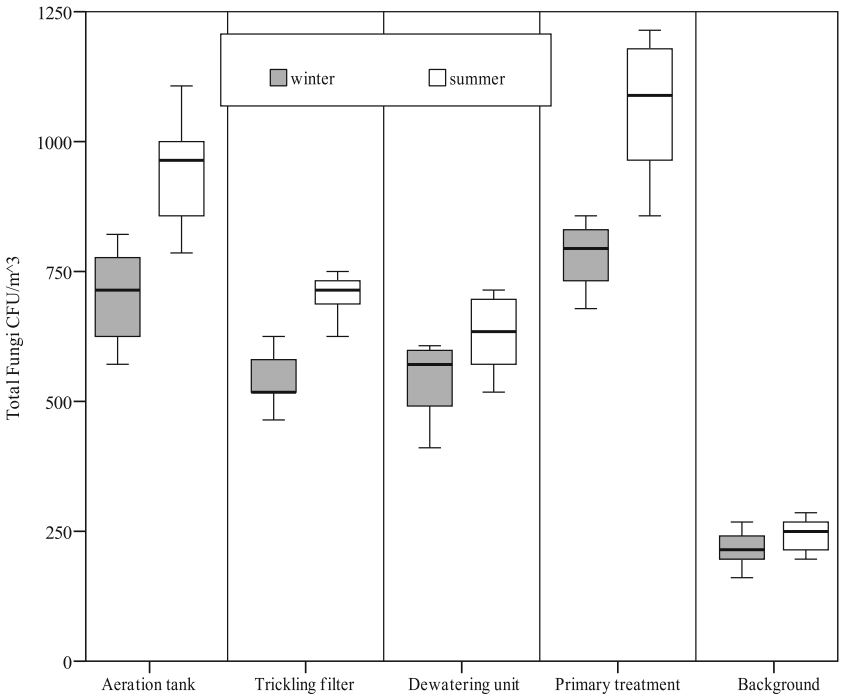


**Fig. 2** Concentration of detected fungi in sampling point in two seasons (winter and summer) as CFU/m$^3$ ($N$=120)

**Table 4** Mean (min-max). The type and spices of detected bacteria as CFU/m$^3$ in five sampling points in winter and summer

| Sampling points | Aeration tank | | Trickling filters | | Dewatering unit | | Primary settling | | Background | |
|---|---|---|---|---|---|---|---|---|---|---|
| Type of bacteria | Winter | Summer | Winter | Summer | Winter | Summer | Winter | Summer | Winter | Summer |
| *Bacillus* | 144 (54–214) | 433 (268–482) | 113 (56–160) | 318 (178–464) | 90 (54–178) | 296 (196–427) | 135 (71–142) | 421 (321–446) | 46 (18–71) | 74 (0–89) |
| *S. aureus* | 138 (71–196) | 329 (232–428) | 95 (36–142) | 253 (178–339) | 71 (35–107) | 208 (107–285) | 113 (53–142) | 290 (232–339) | 43 (0–89) | 61 (18–89) |
| *S. saprophyticus* | 88 (36–107) | 240 (178–303) | 46 (18–71) | 183 (89–285) | 62.5 (35–89) | 167 (125–196) | 85 (71–107) | 253 (107–357) | 25 (0–36) | 62.5 (36–107) |
| *S. epidermidis* | 83 (71–142) | 258 (196–321) | 67 (17–107) | 195 (142–235) | 56 (17–107) | 180 (125–214) | 68 (36–107) | 235 (125–321) | 21 (17–89) | 64 (0–89) |
| *S. muscase* | 7.5 (4–18) | 21 (0–71) | 4.5 (0–18) | 55 (36–71) | 9 (0–18) | 23 (0–53) | 12 (0–53) | 25 (17–71) | 1.5 (0–18) | 7.5 (0–35) |
| *M. agilis* | 97 (71–144) | 228 (107–331) | 80.5 (35–142) | 193 (107–285) | 86 (71–142) | 164 (89–232) | 105 (36–196) | 229 (142–303) | 34 (0–71) | 64 (17–89) |
| *M. rezeus* | 177 (89–267) | 138 (71–196) | 95 (89–178) | 114 (53–214) | 85 (17–160) | 91 (35–196) | 133 (53–232) | 138 (71–160) | 39 (35–71) | 34 (17–53) |
| *M. luteus* | 165 (89–232) | 197 (125–357) | 95 (53–160) | 102 (35–142) | 104 (71–178) | 82 (71–160) | 186 (89–285) | 131 (89–196) | 47 (35–53) | 34 (17–71) |
| *M. nishinomiycesis* | 23 (17–35) | 67 (35–89) | 24 (17–53) | 50 (25–75) | 9 (0–18) | 25 (0–35) | 22 (0–53) | 46 (35–71) | 9 (0–17) | 9 (0–17) |
| *M. sedentarius* | 34 (17–53) | 43 (35–71) | 12 (0–18) | 30 (20–40) | 25 (17–53) | 27 (17–35) | 35 (0–71) | 44 (35–89) | 7.5 (0–17) | 3 (0–9) |
| Gram-positive coci | 10 (0–17) | 39 (35–53) | 2.5 (0–5) | 20 (17–35) | 4 (0–9) | 14 (0–17) | 11 (0–17) | 24 (17–35) | 1.5 (0–17) | 3 (0–9) |
| Gram-positive bacil | 3 (0–6) | 7 (0–9) | 2 (0–4) | 25 (0–53) | 6 (0–9) | 12 (0–17) | 10 (0–17) | 22 (0–53) | 1.5 (0–9) | 1.5 (0–9) |

*S* Staphylococcus *M* Micrococcus

location A, *Bacillus* with emission of 433 CFU/m$^3$, *Micrococcus aureus* with average emission of 329, and *Staphylococcus epidermidis* with average emission of 240 showed the maximum emissions in summer. Moreover, in the winter, *Micrococcus rezeus*, *Micrococcus luteus*, and *Bacillus* spp., were found to be the dominant bacteria types in location A. The average of bacterial emission in other locations is listed in Table 1. Similarly, Michalkiewicz reported *Corynebacterium*, *Bacillus* spp., *Staphylococcus* spp., and *Micrococcus* spp., as the prevalent bacteria (Michałkiewicz et al. 2011). According to Breza, *Pseudomonas* were the prevailing bacteria (Breza-Boruta and Paluszak 2007).

## Airborne fungal identification

The frequency and the prevailing types and species of fungi in winter and summer are shown in Table 5, respectively. According to Table 5, in winter, in all the sampling locations, *Cladosporium* spp. and *Penicillium* spp. were prominent fungal emissions, followed by *Alternaria* spp. and *Mucor* spp. In summer, similarly, *Cladosporium* spp. and *Penicillium* spp. were the dominant types. Also, the emission of *Alternaria* spp., *Ulocladium* spp., and *Aspergillus* spp., were found to be high. Similarly, according to Malecka, the dominant fungal types were: *Penicillium* spp. 54 %, *Aspergillus* spp. 23 %, *Cladosporium* spp. 11 %, *Fusarium* spp. 6 %, and *Alternaria* spp. 3 % (Małecka-Adamowicz et al. 2007). Also, Dutkiewicz found *Penicillium* spp., *Alternaria* spp., and *Aspergillus fumigatus* as the most frequent types (Prazmo et al. 2003). All the observed fungal species have abilities to form spores, which is a protective mechanism against environmental changes. Based on result, the dominant frequency of these types and species could be attributed to their metabolic potentiality which preserves their distribution and survival under unfavorable conditions such as UV radiation, lack of food, and high temperature.

## Seasonal concentration and distribution of bacterial and fungal aerosols

As shown in Table 5, the highest pollution was observed in the warm season. The rate of bacterial emission in the summer was approximately 1.5 times more than that in the winter. This is in agreement with Breza-Boruta and Paluszak 2007, Oppliger et al. 2005, and (Korzeniewska 2011a, b). Also, Fang et al. 2008 and Grisoli et al. 2009 concluded that fungal

**Table 5** Mean (min-max) the type and spices of detected fungal as CFU/m$^3$ in five sampling points in winter and summer

| Sampling points | Aeration tank | | Trickling filters | | Dewatering unit | | Primary settling | | Background | |
|---|---|---|---|---|---|---|---|---|---|---|
| Type of fungi | Winter | Summer | Winter | Summer | Winter | Summer | Winter | Summer | Winter | Summer |
| Cladosporium | 196 (71–303) | 267 (178–357) | 130 (53–214) | 220 (71–446) | 180 (53–285) | 197 (125–375) | 178 (89–267) | 285 (178–464) | 53(35–107) | 71.5(18–89) |
| Penecallium | 125 (35–250) | 214 (89–303) | 92 (53–160) | 170 (71–196) | 127 (17–178) | 107 (89–232) | 143 (125–250) | 214 (146–331) | 53 (35–71) | 53 (0–71) |
| Aspergillus | 28.5 (18–36) | 88 (71–150) | 29 (17–53) | 71 (36–90) | 2 (0–5) | 78 (72–108) | 71 (18–90) | 90 (54–146) | 2 (0–3) | 58 (40–72) |
| Alternaria | 93 (54–107) | 143 (71–196) | 90 (35–196) | 90 (35–171) | 90 (54–107) | 90 (72–164) | 114 (71–212) | 143 (108–196) | 2 (0–4) | 3.5 (0–5) |
| Rhodotorula | 7 (0–10) | 7 (0–11) | 2 (0–5) | 3.5 (0–6) | 32 (18–54) | 7 (2–10) | 53 35–89) | 24.5 (18–71) | 44 (0–89) | 2 (0–3) |
| Trichoderma | 23.5 (18–71) | 9 (0–12) | 44.5 (35–90) | 9.5 (4–18) | 5 (0–18) | 7.5 (0–18) | 35 (18–72) | 9 (0–35) | 0 (0–0) | 1.5 (0–3) |
| Fusarium | 50 (35–89) | 32 (0–90) | 53.5 (35–107) | 38 (0–72) | 35 (0–53) | 27 (17–35) | 60 (35–90) | 50 (35–71) | 18 (0–35) | 0 (0–0) |
| Ulocladium | 35 (0–6) | 89 (72–107) | 26 (0–54) | 71 (35–90) | 28 (0–35) | 71 (35–146) | 2 (0–4) | 125 (107–178) | 35 (0–54) | 30 (18–72) |
| Mucor | 74 (18–90) | 47.5 (0–90) | 76 (54–160) | 30 (0–71) | 41 (35–90) | 3.5 (0–6) | 87 (54–107) | 44.5 (18–71) | 7 (3–10) | 18 (0–35) |
| Rhizopus | 39 (0–72) | 3.5 (0–5) | 2 (0–4) | 7 (0–12) | 2 (0–3) | 2 (0–3) | 1.5 (0–5) | 24 (0–35) | 0 (0–0) | 0 (0–0) |
| Myselium | 33 (0–53) | 33 (18–35) | 3.5 (0–5) | 4.5 (0–6) | 3.5 (0–5) | 57 (35–71) | 35 (18–54) | 31 (0–35) | 0 (0–0) | 1.5 (0–3) |

emission was more in the summer than that in winter. According to Krzysztofic's report, high relative humidity along with high temperature and mild wind lead to the formation of bioaerosols in summer (Krzysztofik 1992).

## Relationship between measured variables and the pollution concentration of samples

The correlation among meteorological condition as well as the relationship between meteorological conditions and concentration of the bacteria and fungi were investigated in sampling days and are given in Table 6. We found a significant correlation between concentration of detected bacteria and temperature, UV index, and wind speed. Based on these results, temperature seems to have the highest correlation with bacteria concentration ($R$=0.613, $P$ value <0.001, $N$=120), Also, a significant correlation was seen between concentration of fungal and relative humidity. Significant correlation between concentration of detected bacteria and atmospheric pressure, relative humidity was not found. In addition, the correlation between fungal concentration and wind speed, pressure, and UV index was not seen. To identify which parameters have strongest effect on bioaerosol level, all parameters were considered simultaneously using the multiple regression model based on the standardized coefficients; temperature has the greatest influence on the emission bacteria;

**Table 6** Correlation between the meteorological conditions and total bacterial and fungal concentration in the investigated samples ($N$=120)

| | Temperature | Humidity | UV index | Pressure | Wind speed | Total bacteria | Total fungal |
|---|---|---|---|---|---|---|---|
| Temperature | 1 | | | | | | |
| Humidity | −0.585** | 1 | | | | | |
| UV index | 0.856** | −0.439* | 1 | | | | |
| Pressure | −0.834** | 0.245 | −0.740** | 1 | | | |
| Wind speed | 0.697** | −0.511* | 0.475* | −0.457* | 1 | | |
| Total bacteria | 0.613** | −0.352 | 0.598** | −0.325 | 0.591** | 1 | |
| Total fungal | 0.448* | 0.680** | 0.149 | 0.215 | 0.129 | 0.655** | 1 |

Significant: **$P$<0.01 and *$P$<0.05

however, the effect was not statically significant (Beta=0.806, *P* value 0.209). Also, humidity has the strangest statistically significant effect on the emission fungal spores (Beta=0.822, *P* value=0.003).

Oppliger et al. 2005 showed that meteorological parameters clearly affected fungal concentration. Breza et al. found that there was a significant relationship between meteorological parameters and rate and type of bacteria (Breza-Boruta and Paluszak 2007). In the contrary, Carducci did not find any significant relationship between meteorological parameters and the number of bacterial colonies (Carducci et al. 2000).

## Conclusion

Our findings show maximum bacterial concentration was found in the aeration tank with an average of 1973 CFU/$m^3$ in the summer and 1016 CFU/$m^3$ in the winter. Also, minimum bacterial concentration was observed in the sludge dewatering unit with an average of 1301 and 602 CFU/$m^3$ in the summer and winter, respectively. Maximum and minimum fungal concentrations were in primary treatment and sludge dewatering unit with an average of 781 and 1063 CFU/$m^3$ in winter and summer, respectively. In general, the emission of bacterial and fungal bioaerosol was high and could be concluded that they were at hazard levels of bioaerosol. *Bacillus*, *Staphylococcus* spp., *and Micrococcus* spp. were the most frequently observed bacteria types in the WWTP. *Micrococcus* spp. and *Staphylococcus* spp. resulted to be the highest emission in the winter and summer, respectively, followed by *Bacillus* spp. One possible reason for this condition is that these species are resistant in unfavorable conditions. The dominant fungi were *Cladosporium* spp. and *Penicillium* spp., followed by *Aspergillus* spp. and *Alternaria* spp. There was a significant relationship between environmental parameters and concentrations of bacterial and fungal.

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